Food safety

Dr. Mostafa Noroozi (PhD)

Associated professor of nutrition

Health Risks of GM Food?

Can Science Give us The Tools for Recognizing Possible...

Health Risks of GM Food?

قوانین ایمنی زیستی

BIOSAFETY LAW

Without labeling,
it's impossible to know
if you are eating GM food.
Does that account
for the lack of
reported sickness?

Risk Assessment Process

The hazard identification process reveals that there are three principal issues that merit further risk assessment.

1. The safety of the inserted DNA
2. The safety of the newly introduced component
3. In the food safety evaluation of GM foods specific questions that must be answered should be:
4. Is the transferred DNA safe to consume?
5. If an antibiotic resistance marker is used, is it safe?

Risk Assessment Process

1. Are the newly produced proteins safe to consume?
2. Have potential allergens been introduced into the food?
3. Are the composition and nutritional value changed?
4. Are there changes in the content of important substances?
5. In what forms will the food or food products isolated from it be consumed?
6. What is the expected human dietary exposure?

ارزیابی ایمنی محصولات کشاورزی تراریخته

فاز اول

آنالیز تفاوت میان محصولات کشاورزی تراریخته و غیرتراریخته
(کلیدی اصل این مبحث)

متابولیت های ثانویه (جدید)

پروتئین های بیان شده (جدید)

ژن های القاء شده

فاز دوم

مطالعات سم شناسی و تغذیه ای تفاوت های شناسایی شده در محصولات کشاورزی تراریخته

آزمون های سم شناسی بیشتر - در صورت نیاز - ممکن است بر روی غذای کامل

برآورد مقدار دریافتی

سم شناسی

قابلیت دسترسی زیستی

ویژگی های تجزیه ای

حساسیت زایی

انتقال ژن

فاز سوم

ارزیابی سم شناسی و تغذیه ای

فاز چهارم

ارزیابی ایمنی نهایی محصولات کشاورزی تراریخته

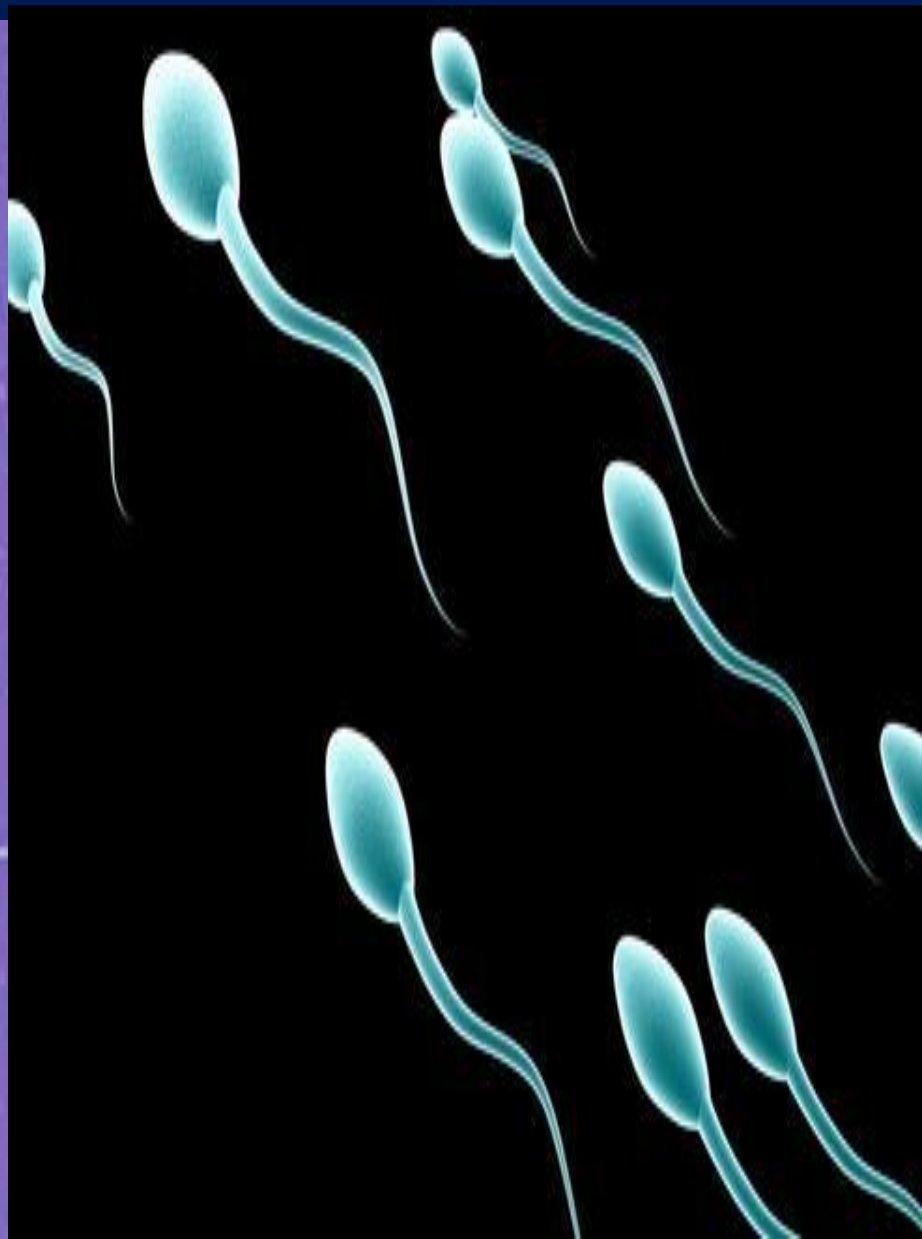
Measurement of oxidative stress and genotoxicity methods in foods

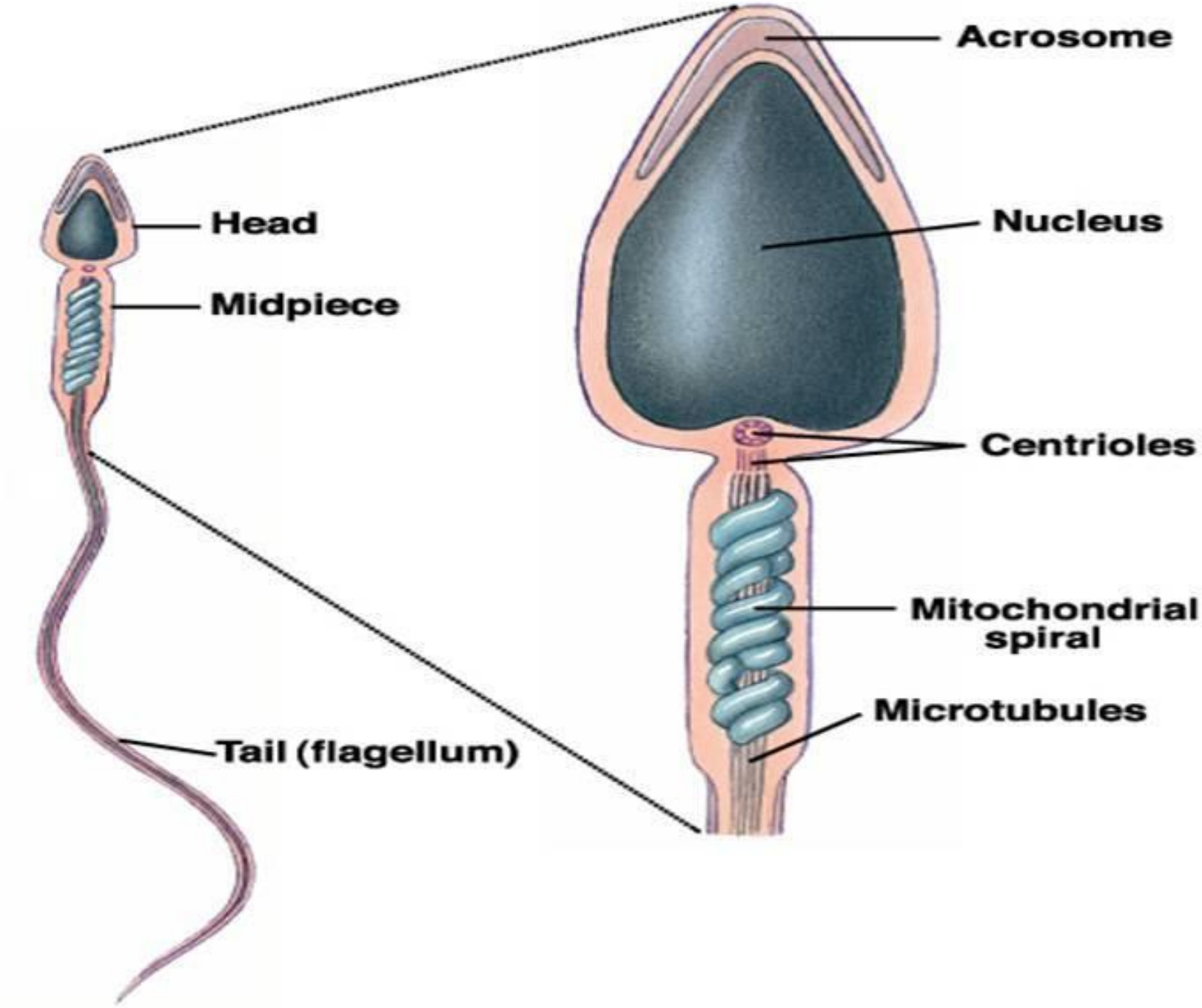
1. Ames test
2. SCGE test (Single cell gell electrophoresis)
3. Endonucleas III test

SCGE test (Single cell gell electrophoresis)

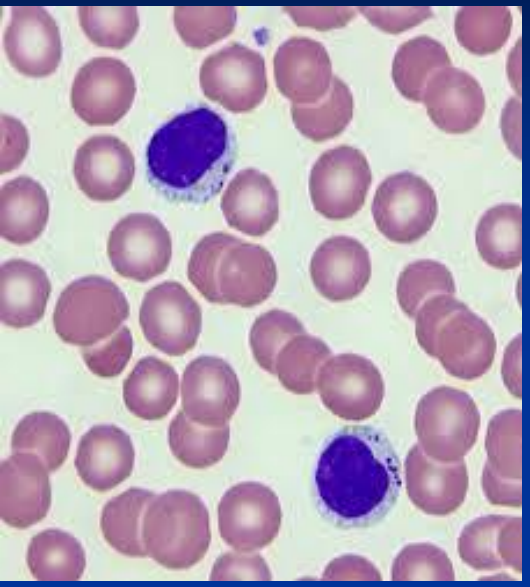
SCGE test (Single cell gell electrophoresis)

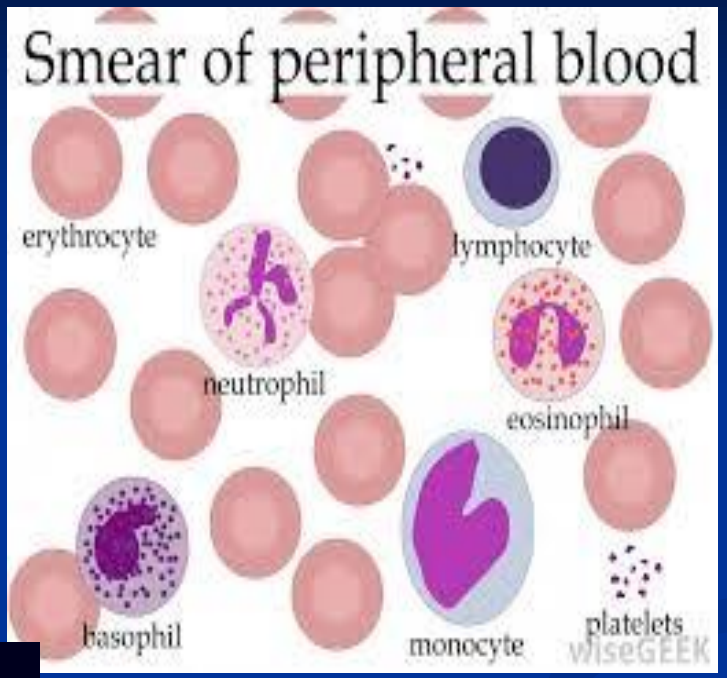
SCGE test (Single cell gell electrophoresis)

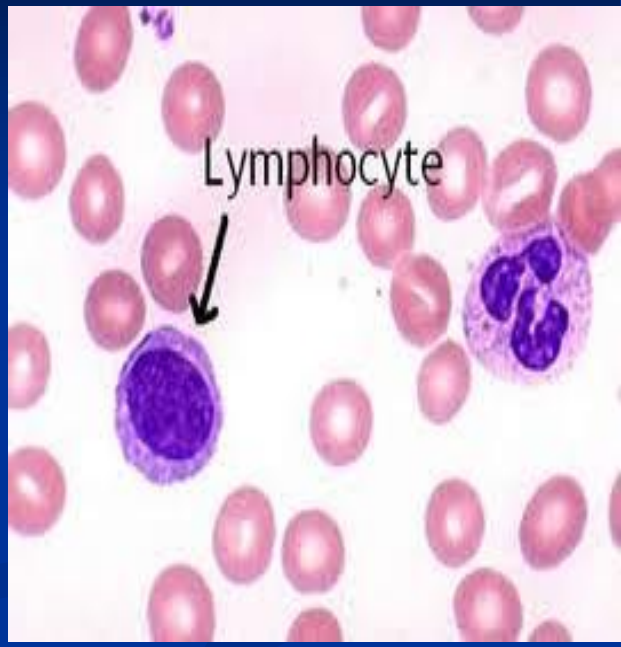


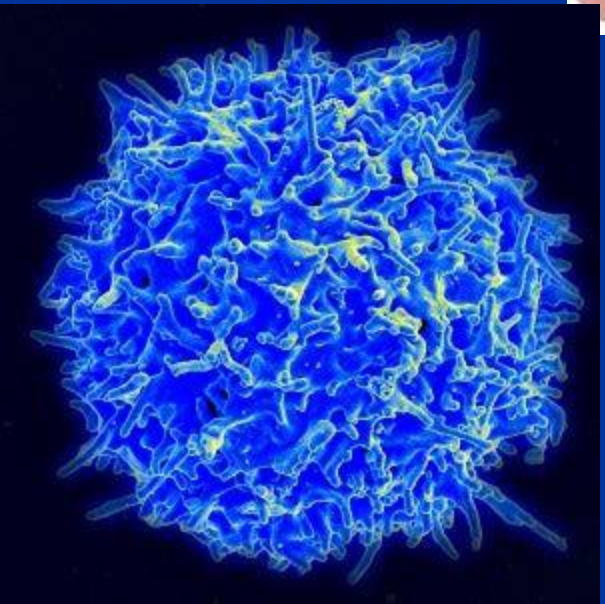

Acrosome
Head
Nucleus
Midpiece
Centrioles
Mitochondrial spiral
Microtubules
Tail (flagellum)

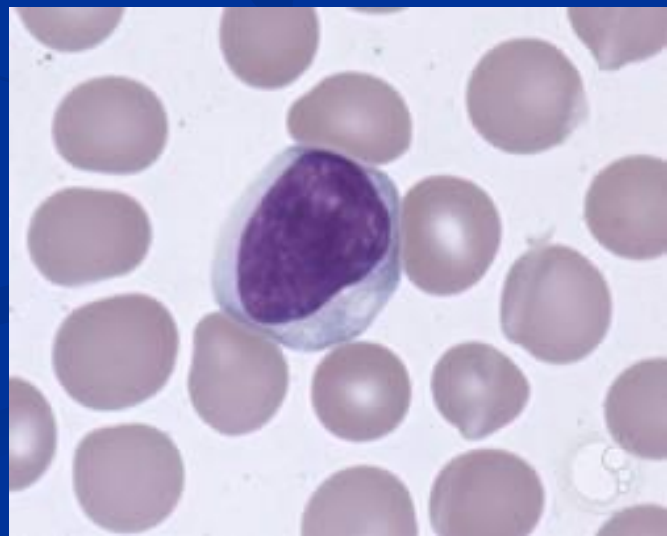
T- lymphocytes

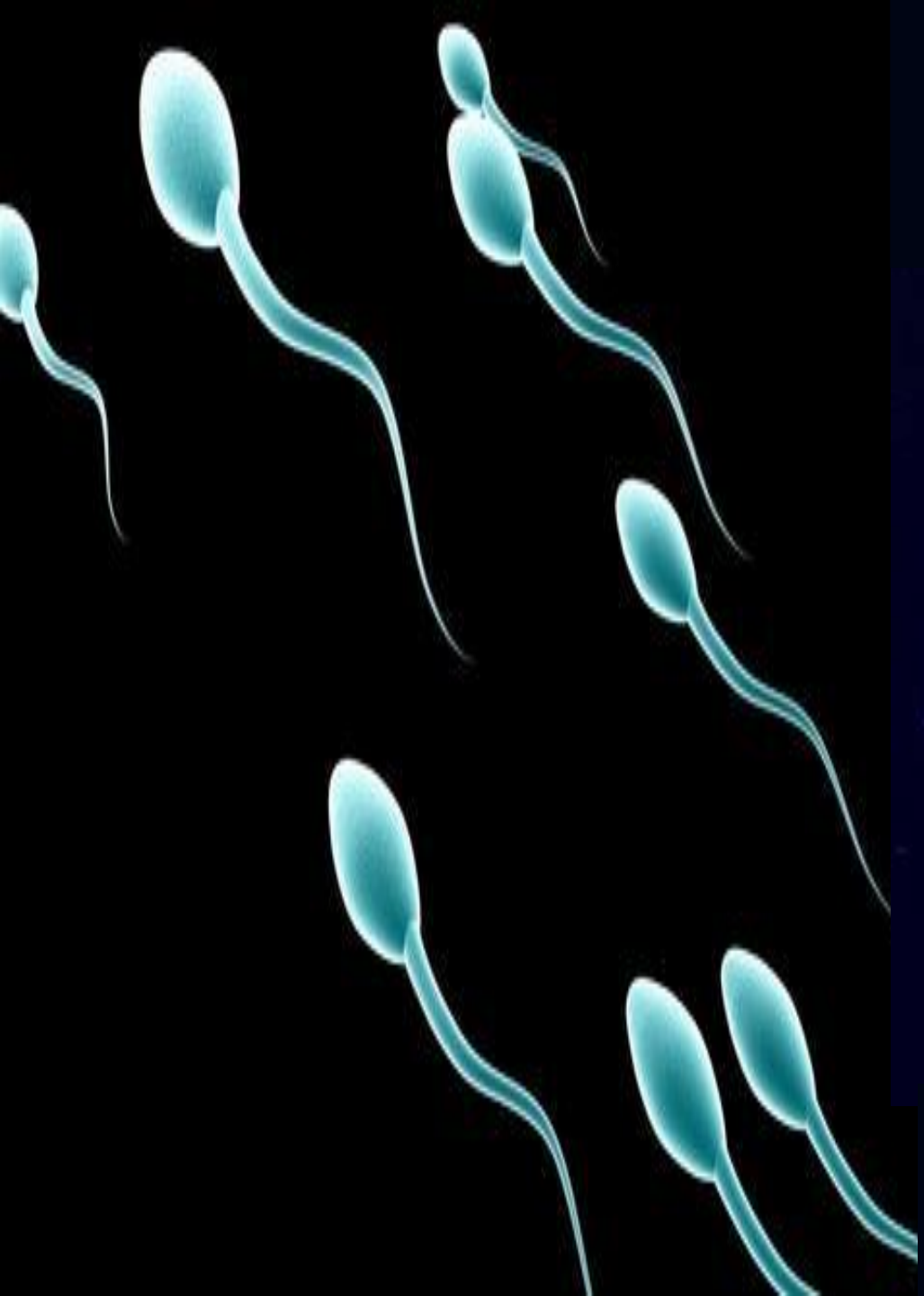


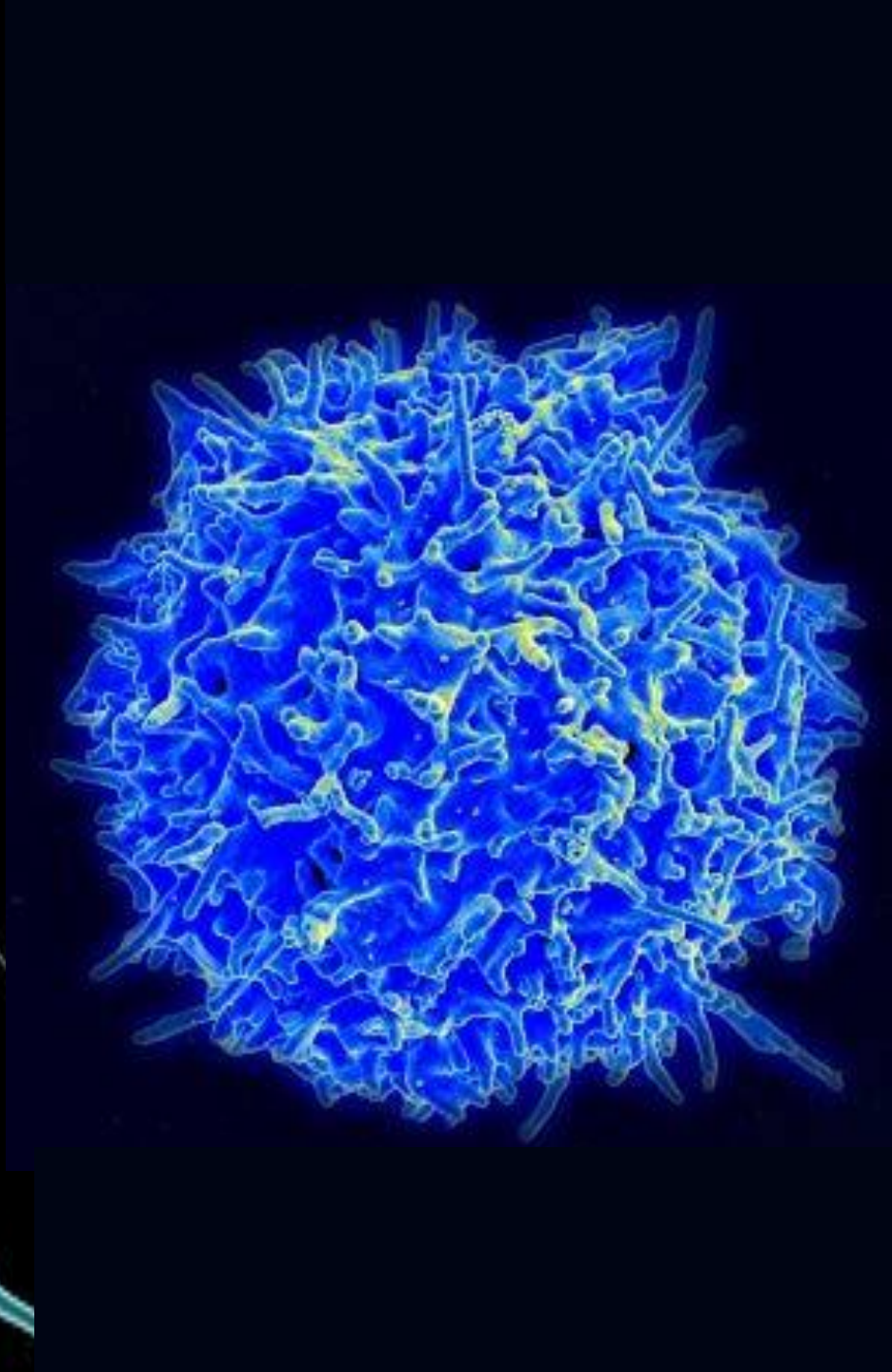












Chemicals, solutions and materials

The solutions and chemicals used in this study were purchased from the following companies:

- High melting point agarose (HMP) and low melting point agarose (LMP) both electrophoresis grade from Gibco Ltd., Paisley, Scotland;
- Phosphate-buffer saline (PBS) tablets,
- ethylenediamine tetraacetic acid disodium salt dihydrate (EDTA Na2), trypan blue solution (0.4%), all from Sigma Chemicals Co. Ltd., Irvine, Scotland.

Chemicals, solutions and materials

- RPMI (1640) medium with NaHCO3 without L-glutamine and phenol red,
- Histopaque-1077,
- ethidium bromide (EtBr)
- diamidine-2-phenylindol dihydrochloride (DAPI)

all from Sigma Chemicals Co. Ltd., Irvine, Scotland; foetal calf serum (FCS) from Globe Pharm Ltd., Esher, Surrey, England;

- sodium hydroxide, hydrogen peroxide,
- Triton X-100, sodium chloride from BDH Chemicals Co. Ltd., England;
- Tris from Boehringer, Mannheim Ltd., Sussex, England;
- Fully frosted Dakin microscope slides were supplied by Richardson Supply, London, England.

Cell preparation

Human lymphocytes were isolated from fresh whole blood by adding 30 μL blood to 1 mL RPMI 1640 with 10% FCS on ice for 30 min, then underlaying it with Histopaque 1077 before centrifuging at 200 × *g* for 3 min at 4 °C. Lymphocytes were separated as a pink layer at the top of the Histopaque 1077.

Antioxidant pretreatment

Cells were incubated with different concentrations of flavonoids or vitamin C for 30 min at 37 °C in a dark incubator together with untreated control samples. Samples were then centrifuged at 200 × *g* for 3 min at 4 °C. After pretreatment, cells were centrifuged and washed twice with PBS (0.01 mol) at 200 × *g* for 3 min at 4 °C.

Oxygen-radical treatment

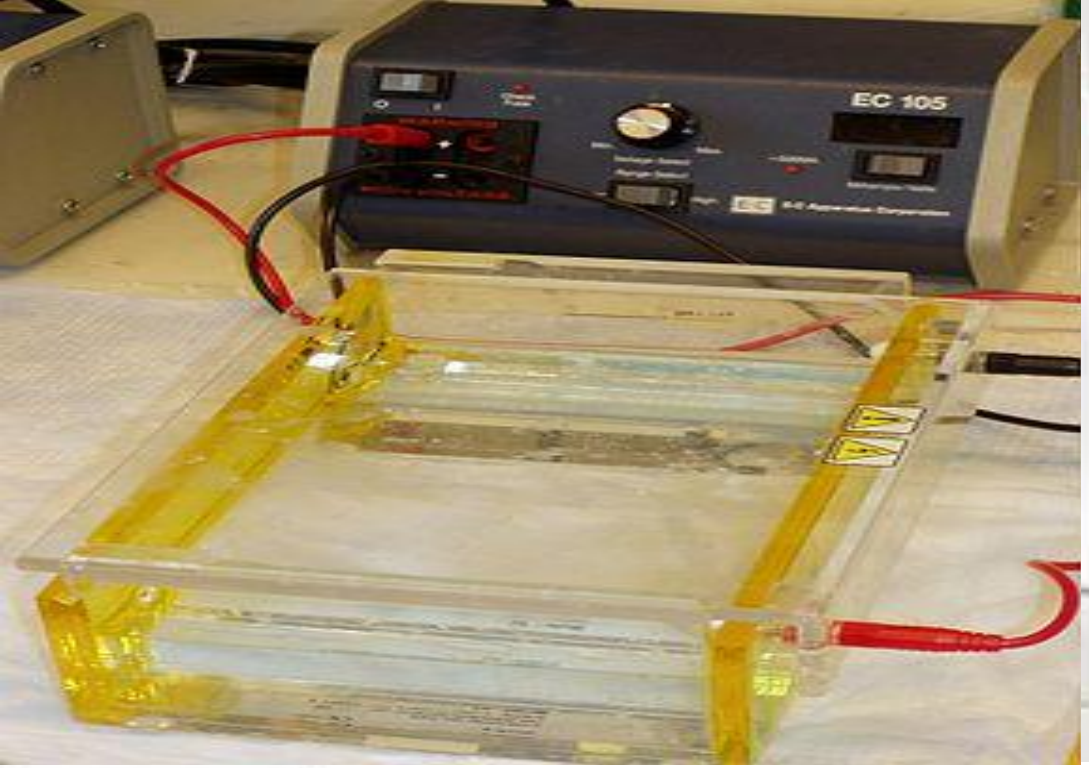
Samples were suspended in PBS with 100 μmol H_2O_2/L for 5 min on ice in the dark. Samples were then centrifuged at 200 × *g* for 3 min at 4 °C. Control samples were treated with PBS alone without hydrogen peroxide.

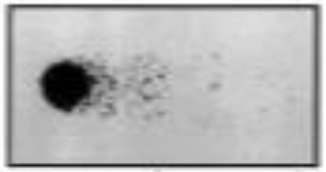
Slide preparation

Two layers of agarose were prepared. For the first layer, 85 μL 1% HMP agarose or standard agarose prepared at 40 °C in PBS was dispensed onto fully frosted slides and covered with a 22 × 22 mm (no. 1) coverslip. To solidify the agarose, the slides were kept at 4 °C for 10 min. Lymphocytes were suspended in 1% LMP agarose in PBS (prepared at 37 °C) and 85 μL containing ≈20 000 lymphocytes were plated onto the first layer of agarose, covered with a coverslip, and kept for 10 min at 4 °C to solidify. After the coverslips were removed, the slides were immersed in freshly prepared cold lysing solution.

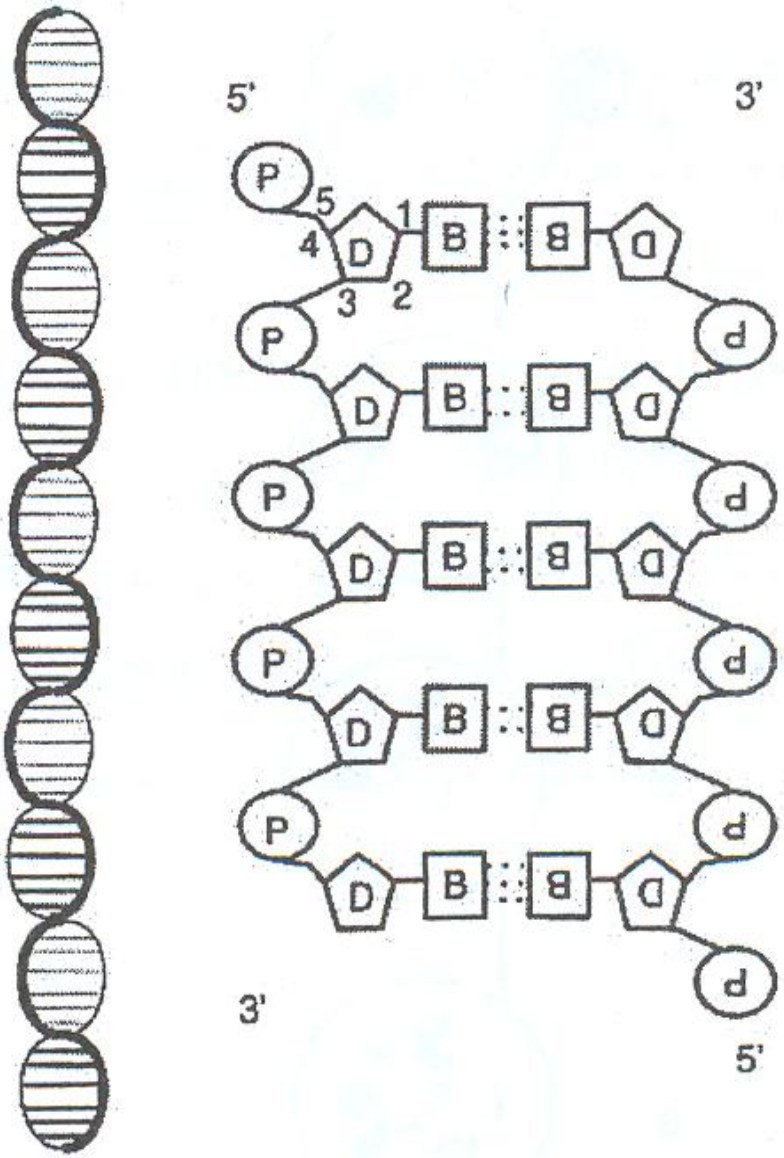
Alkaline treatment and electrophoresis

After the slides were removed from the lysis solution they were placed in an electrophoresis tank horizontally, side by side. Up to 18 slides, in two rows of 9, were electrophoresed simultaneously. Any gaps were filled with blank slides to avoid spaces between slides. Slides were covered with fresh electrophoresis buffer (300 mol NaOH/L and 1 mol Na_2EDTA/L, pH 13) at 4 °C for 40 min to a depth of 2–3 mm above the slides. Buffer was made freshly each day and stored at 4 °C before use. To prevent additional DNA damage, slides were processed in dark conditions. The electrophoresis was run at 25 V for 30 min at 4 °C, covered with black paper against light.

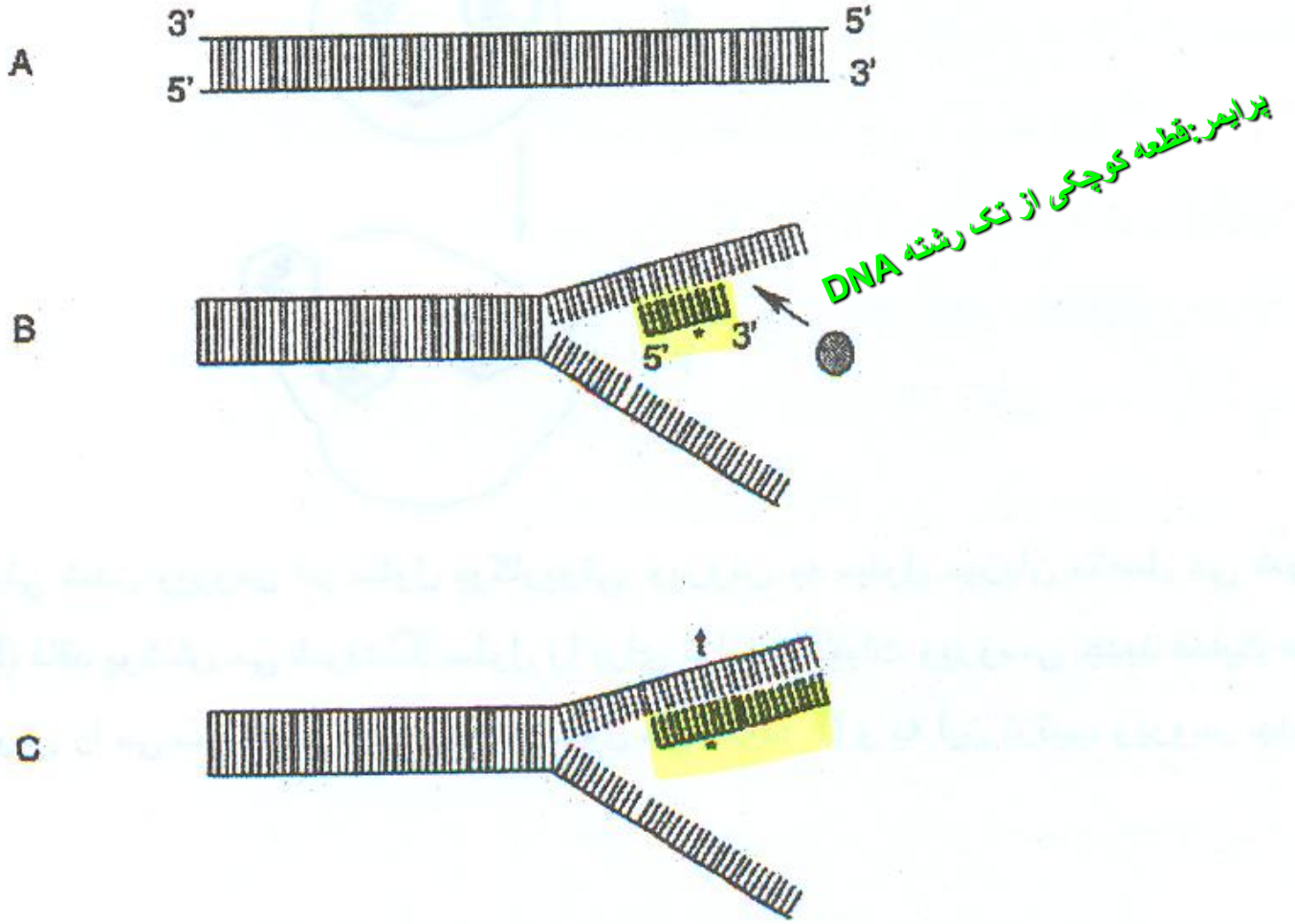




CELLS → SLIDES → LYSIS → ALKALI UNWINDING (pH >13, Duration ?) → ELECTROPHORESIS (V/cm ? , mA ? , pH >13) → NEUTRALIZATION → STAINING

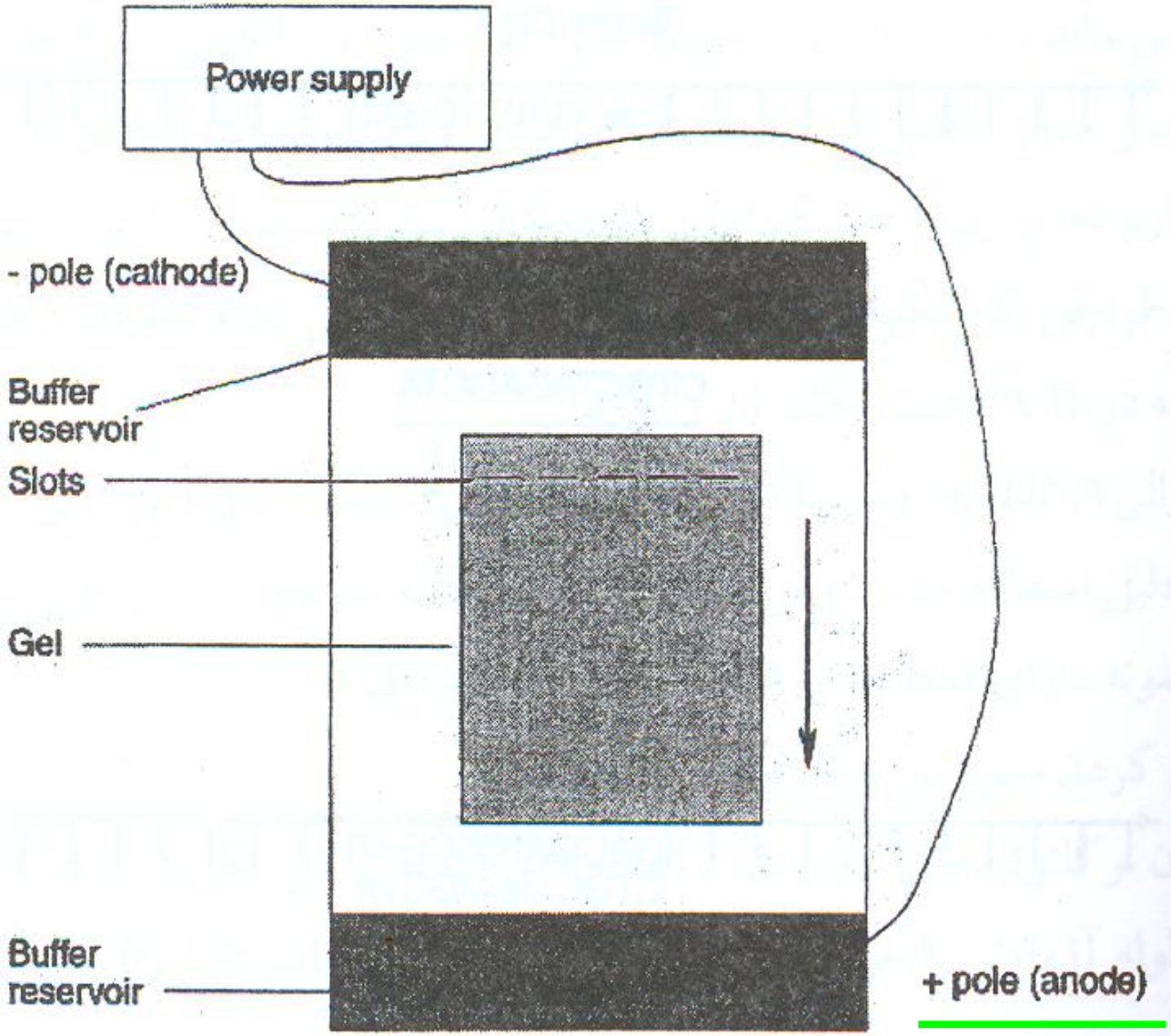


شکل۹-۲: ساختمان DNA . ساختار اصلی از فسفات (P) و داکسی ریبوز (D) ساخته شده است .بازها از طریق
انتهای هیدروژنی به همدیگر متصل می شوند (B).



پرایمر: قطعه کوچکی از تک رشته DNA

همانند سازی DNA. DNA دو رشته ای (A) به رشته های منفرد تبدیل می شود. پرایمر (*) به یکی از رشته های منفرد متصل می شود (B) و DNA پلیمراز (دایره پر شده) رشته جدید را گسترش می دهد. (C) عمل شروع همانند سازی از پرایمر صورت می گیرد و بازهای مکمل به رشته الگو (t) اضافه می شوند. رشته اصلی دیگر نیز به عنوان الگو عمل می کند.



شکل ۱۳-۲: ژل الکتروفورز. ژل در بافر غوطه ور شده و قطعات DNA به طرف آن حرکت می کنند (که بوسیله پیکان مشخص شده است).

Quantification of DNA damage

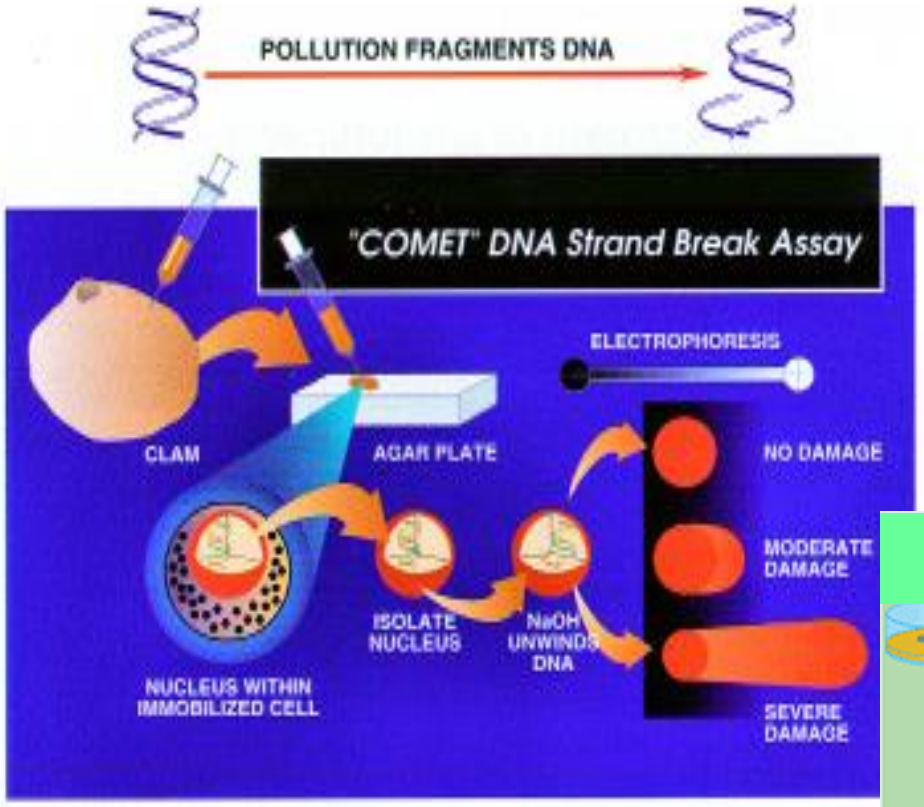
Slides were examined at 400× magnification on an Olympus fluorescence microscope (Olympus Optical Co, Ltd, Tokyo) with excitation at 520 nm and a 620-nm emission barrier filter. Because the study involved the individual assessment of DNA damage in > 30 000 cells, it was necessary to develop a rapid visual scoring system. Cells were assigned a score on a 5-point scale (range: 0–4) according to the amount of DNA in the tail of the comet as estimated by the observer (Figure 1). To validate this system, objective measurements of the distribution of DNA were performed for a sample of cells by using a BRS2 Image Analyser (Imaging Research, Inc). These measurements were made by quantifying the fluorescent intensity distribution of the comet as a function of distance from the leading edge of the head

Slide scoring

Randomly selected lymphocytes were graded visually for each slide. A total damage score for the slide was derived by multiplying the number of cells assigned to each grade of damage by the numeric value of the grade and summing over all grades (giving a maximum possible score of 400, corresponding to 100 cells of grade 4).

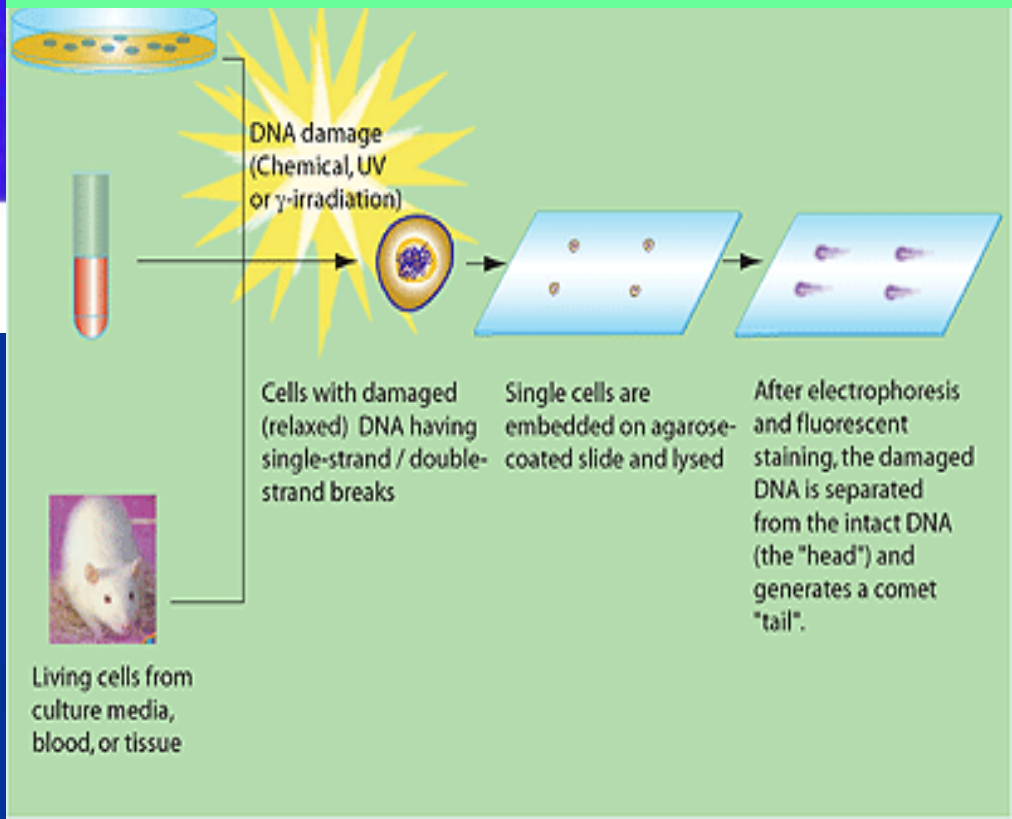
In a given experiment, duplicate slides were prepared and scored for each concentration of the antioxidant. Experiments were repeated three to six times. Therefore, for each concentration of each antioxidant, 6–12 samples of 100 randomly selected cells were analyzed in total.

2- SCGE test (Single cell gell electrophoresis)



The Comet Assay:
A method for the detection of the genotoxic and cytotoxic effects of pollutants.

A genotoxicological technique for measuring DNA damage in an individual cell using single-cell gel electrophoresis.



Comet Assay Procedure

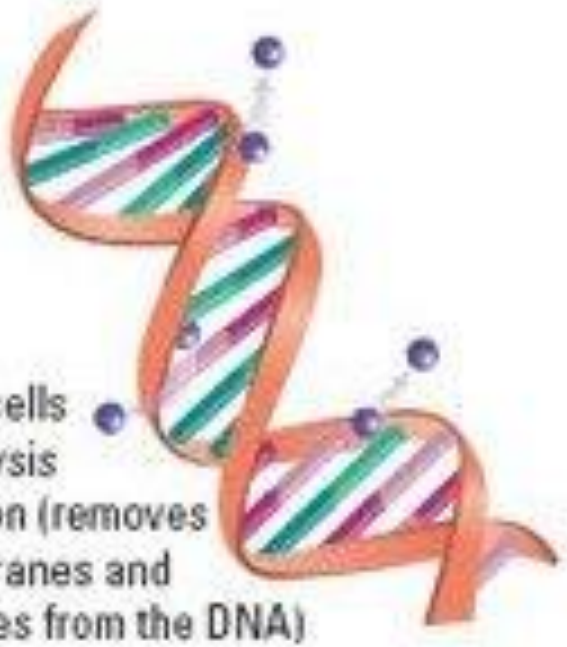
1

Cells mixed with low melting agarose at 37°C (LM Agarose)

2

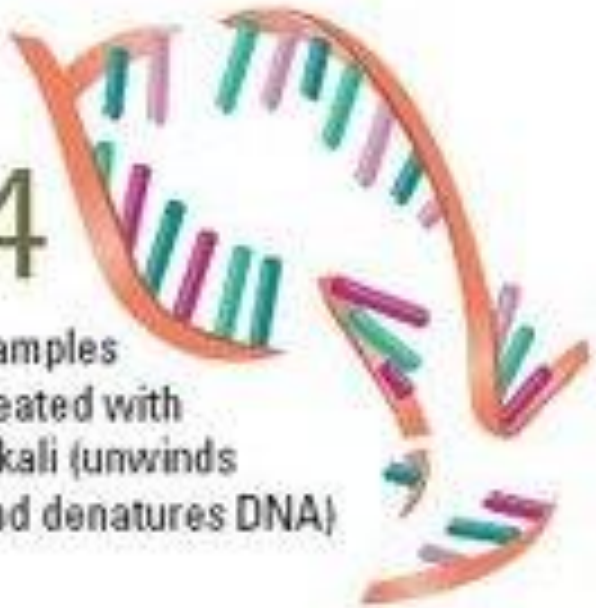
Immobilize cells on CometSlide™

3



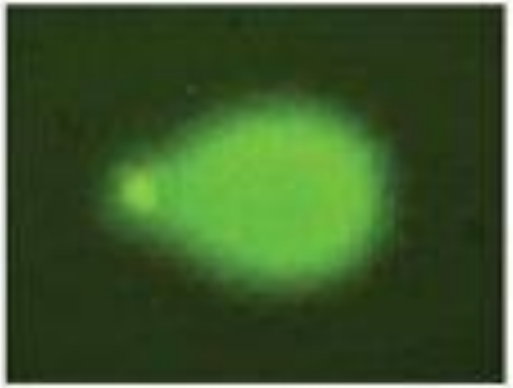
Treat cells with Lysis Solution (removes membranes and histones from the DNA)

4

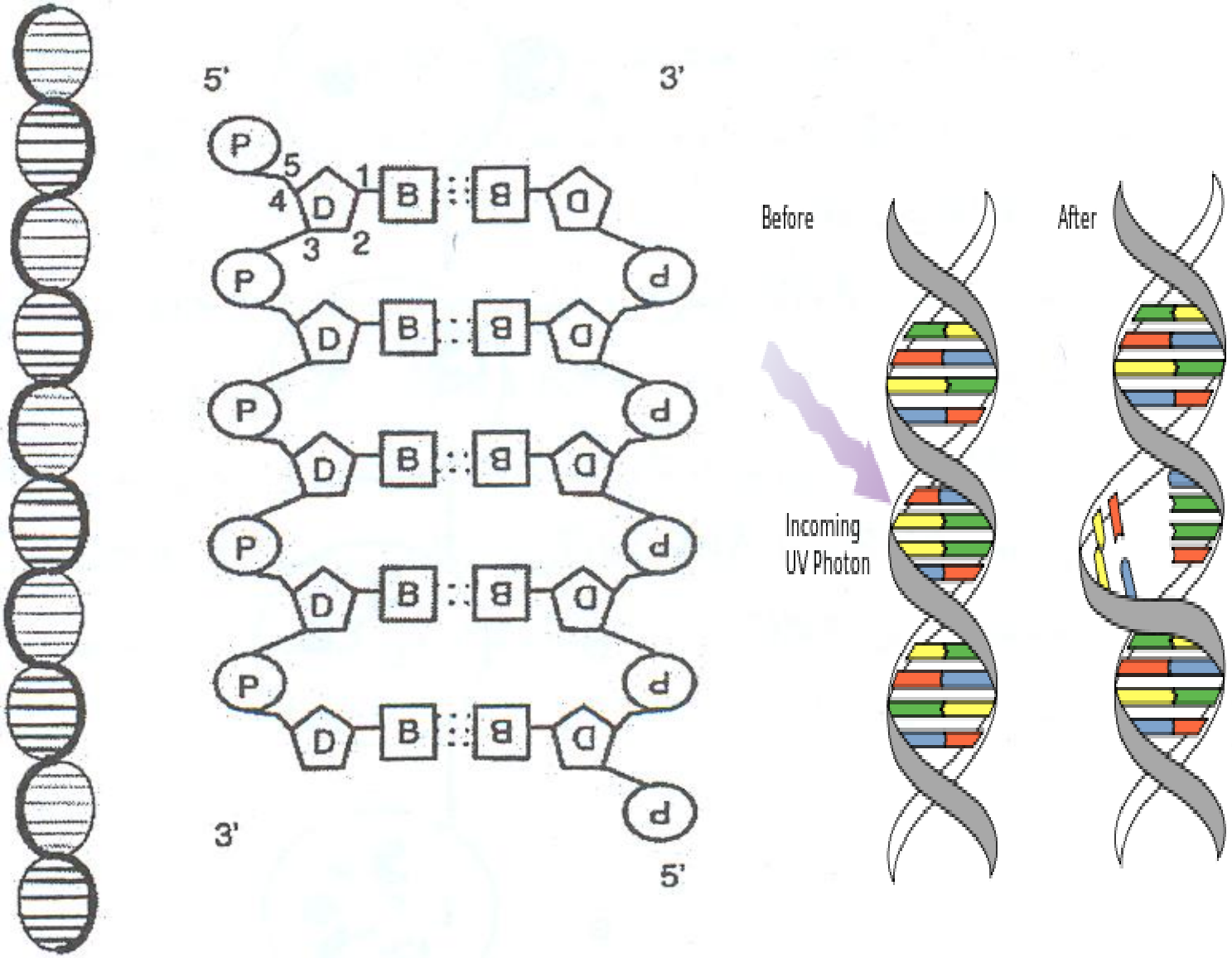


Samples treated with alkali (unwinds and denatures DNA)

5



Samples stained with intercalating dye and visualized by epifluorescence microscopy following alkaline electrophoresis, which reveals DNA breaks



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انتهای هیدروژنی به همدیگر متصل می شوند (B).